RNA PowerSoil™
Total RNA Isolation Kit*

Catalog No.	Quantity
12866-25	25 Preps

Instruction Manual

Please recycle

***PATENT PENDING**

Version: 09162008

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Introduction

MO BIO Laboratories' RNA PowerSoil™ Total RNA Isolation Kit is designed to isolate total RNA from organisms found in soil. The patent pending properties of the kit permit consistent removal of humic substances, fulvic acids, and other RT-PCR inhibitors from soil purified RNA. Diverse soil types, including compost, manure, estuary sediment, and other soil types high in organic content, have successfully provided biologically intact and RT-PCR amplifiable RNA using this kit. The RNA PowerSoil™ Total RNA Isolation Kit reliably provides RNA for experiments requiring qualitative and quantitative RT-PCR analysis.

Protocol Overview

The dynamics of microbial diversity in soils will vary with the microbial populations and their metabolic status. This in turn is a function of soil composition, soil moisture content, the amount of sunlight, the availability of nutrients and other environmental factors. The RNA PowerSoil™ Total RNA Isolation Kit is designed to extract RNA from the total soil microflora and microfauna, including metabolically active, metabolically dormant and dead organisms. As such, the RNA PowerSoil™ Total RNA Isolation Kit will provide the best possible representation of the population of soil organisms and RNA composition.

This kit is for research purposes only. Not for diagnostic use.

Other Related Products	Catalog No.	Quantity
RNA PowerSoil™ DNA Elution Accessory Kit	12867-25	25 preps
UltraClean™ Agarose, Molecular Biology Grade	15003-50 15003-100 15003-500 15003-1000	50 g 100 g 500 g 1 kg
UltraClean™ PCR Clean-Up Kit	12500-50 12500-100 12500-250	50 preps 100 preps 250 preps

New!

Try our new RNA PowerSoil™ DNA Elution Accessory Kit (MO BIO Catalog # 12867-25). When used in combination with the RNA PowerSoil™ Total RNA Isolation Kit (MO BIO Catalog # 12866-25) both the RNA and the DNA can be isolated and eluted in two separate fractions.

IMPORTANT: This kit requires user provided phenol:chloroform:isoamyl alcohol (25:24:1, pH 6.5 – 8.0). This reagent can be purchased from Amresco, Incorporated or VWR International (see List of Recommended Vendors for Phenol:Chloroform:Isoamyl Alcohol in the Additional Information Section). The phenol:chloroform:isoamyl alcohol may also be user made (please refer to, Preparing Phenol:Chloroform:Isoamyl Alcohol in the Additional Information Section for preparation instructions). NOTE: Phenol and phenol:chloroform:isoamyl alcohol are subject to oxidation reactions that cause them to become yellow or pink colored, which serves as an indicator that the phenol is NOT useable for RNA extraction. Using colored phenol or colored phenol:chloroform:isoamyl alcohol will result in quality compromised RNA. Prior to each use, a sample of the phenol:chloroform:isoamyl alcohol should be placed in a clear container and its clarity determined. When not in use, store the phenol:chloroform:isoamyl alcohol at 4°C in the dark. Securely cap when not in use and do not expose to light for prolonged periods.

Required Equipment:

Centrifuge capable of centrifuging 15 ml tubes (2500 x *g* minimum)
Microcentrifuge (13,000 x *g*)
Pipettor (20 µl to 1000 µl)
Serological pipettes (1 ml and 10 ml)
Heat block (set at 45°C) (optional)
Vortex
RNase-Free gloves (MO BIO Catalog # 1555-S (small), 1555-M (medium) and 1555-L (large)
Lab Cleaner for RNase Removal (MO BIO Catalog # 12095-250, 12095-500)
Vortex Adapter (MO BIO Catalog # 13000-V1-15 for Vortex Genie 2 or 13000-LV2-15 for Labnet Vortex)

Reagents Required but not Included

Phenol:Chloroform:Isoamyl Alcohol Solution

Kit Contents

	Kit Catalog# 12866-25	
Component	**Catalog #**	**Amount**
Bead Tubes (with 1.5 g beads)	12866-25-PBT	25
Bead Solution	12866-25-BS	69 ml
Solution SR1	12866-25-1	7 ml
Solution SR2	12866-25-2	22 ml
Solution SR3	12866-25-3	42 ml
Solution SR4	12866-25-4	165 ml
Solution SR5	12866-25-5	110 ml
Solution SR6	12866-25-6	28 ml
Solution SR7	12866-25-7	3 ml
RNA Capture Columns	12866-25-SF	25
15 ml Collection Tubes	12866-25-T1	100
2.2 ml Collection Tubes	12866-25-T2	25

Kit Storage

Kit reagents and components should be stored at room temperature (15-30°C).

Precautions

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water. Do not ingest. See Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS information is available upon request (760-929-9911) or at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

WARNING: Wear gloves, laboratory coat and safety glasses when handling phenol. Phenol:chloroform:isoamyl alcohol is a caustic organic solution. User should review the vendor provided MSDS and accident procedures for this reagent. Do not inhale vapors. Follow local ordinances for disposal of phenol waste. Phenol is highly corrosive and can cause severe burns. Chloroform is a carcinogen. In the event of an accident, seek medical attention immediately.

IMPORTANT NOTES FOR USE: Preparing Phenol:Chloroform:Isoamyl Alcohol

Note: Warning: Phenol and Chloroform are organic compounds that are toxic and potentiall dangerous to your health and safety. Please see all manufacturers warnings and precautions before working with these compounds. Phenol and phenol:chloroform:isoamyl alcohol are subject to oxidation reactions that cause them to become yellow or pink colored, which serves as an indicator that the phenol is NOT useable for RNA extraction. Using colored phenol or colored phenol:chloroform:isoamyl alcohol will result in quality compromised RNA. Prior to each use, a sample of the phenol:chloroform:isoamyl alcohol should be placed in a clear container and its clarity determined. When not in use store the phenol:chloroform:isoamyl alcohol at 4°C in the dark. Securely cap when not in use and do not expose to light for prolonged periods.

Preparing Phenol:Chloroform:Isoamyl Alcohol Solution

Mix 25 parts purified phenol, 24 parts chloroform, and one part isoamyl alcohol. This solution can be stored under TE buffer (10mM Tris, 1mM EDTA, pH 8.0) or 0.1M Tris, pH 8.0, for periods up to 3 months at 4°C. Store in an amber bottle to protect from light. It is recommended, if storing under TE Buffer, to add a small volume of a Tris buffer to this solution.

NOTE: Chloroform is mixed with phenol to increase the efficiency of nucleic acid extractions by reducing losses of DNA and RNA at the phenol:aqueous interphase. Chloroform denatures proteins and aids in the removal of lipids, while isoamyl alcohol reduces foaming during the extraction and facilitates the separation of the aqueous and organic phases.

To make Purified Phenol (a component of final solution)

Commercial, liquefied phenol may be used for nucleic acid extraction without redistillation if the phenol is colorless. Crystalline or liquefied phenol that is yellow or pink is not suitable for RNA isolation and will result in compromised RNA quality. Colored phenol may be redistilled at 160°C with the proper laboratory equipment and safety precautions to remove contaminants that cause breakdown or cross linking of RNA and DNA. Unbuffered liquefied or redistilled phenol should be stored frozen at -15 to -25°C in aliquots until needed. Crystalline phenol may be used if the crystals are white and the phenol buffer is equilibrated as described below:

1. Remove phenol aliquots from the freezer and allow to warm to room temperature.
2. Liquefy phenol by immersing in a water bath at 68°C. (NOTE: 8-hydroxyquinoline may be added to a final concentration of 0.1%. This yellow compound is an antioxidant, a partial inhibitor of RNase, and a weak chelator of metal ions. Because the yellow coloring of the 8-hydroxyquinoline will mask phenol color changes due to oxidation, replace the phenol containing 8-hydroxy-quinoline after approximately 6 months storage.) Alternatively, liquefy crystalline phenol by mixing it with an equal volume of 1.0 M Tris-HCl, pH 8.0, and immersing the container in a water bath at 68°C.
3. Transfer the liquefied phenol to a container that will accept a volume equal to the phenol volume.
4. Add an equal volume of 1.0 M Tris-HCl, pH 8.0, invert to mix and allow to stand until a clearly defined phenol:aqueous interphase forms.
5. Remove a sample of the upper aqueous phase and determine the pH.
6. Transfer the remaining upper aqueous phase into an organic waste container.
7. Repeat steps 4 and 5 until the recovered aqueous pH is less than or equal to 8.0 (NOTE: pH greater than 8.0 will not efficiently remove nucleic acid contaminating substances from soil.).
8. Add an equal volume of 0.1M Tris (pH 8.0), invert to mix and allow to stand until a clearly defined phenol:aqueous interphase forms.
9. Remove the 0.1M Tris (8.0) layer and add an equal volume of TE Buffer.
10. Store the equilibrated phenol for up to 3 months at 2-8°C, protected from light in an amber glass bottle.

Determining the pH of Phenol

Accurate pH measurements of organic phenol and phenol:chloroform can be difficult to achieve. Standard reference electrodes measure the liquid junction potential between the electrode's potassium chloride filling solution and the sample. Organic liquids such as phenol and chloroform have very low dielectric constants compared to water. A very large liquid junction potential, present with phenol, can cause problems such as pH drift, long stabilization times and damage to the pH electrode. Because of this, pH paper has often been used to measure the pH of phenol solutions; however, phenol destroys the indicator chemical of the pH paper, resulting in inaccurate pH measurement.

To accurately measure the pH of saturated phenol, it is necessary to solublize the phenol in an aqueous medium. The following methods are used to determine pH of phenol solutions:

Phenol:chloroform:isoamyl alcohol: Mix 2 ml of the organic phase with 8 ml of methanol and 10 ml of water. Measure the pH of the entire sample.

Saturated phenols: Mix 2 ml of the organic phase with 5 ml of methanol and 13 ml of water. Measure the pH of the entire sample.

List of Recommended Vendors for Phenol: Chloroform: Isoamyl Alcohol

Vendor Name	Chemical Name	Catalog Number	Volume (ml)
Amresco, Incorporated*	Phenol: Chloroform (pH 6.7/8.0) 25:24:1 premixed with isoamyl alcohol	0883-100	100
Amresco, Incorporated*	Phenol: Chloroform (pH 6.7/8.0) 25:24:1 premixed with isoamyl alcohol	0883-400	400
VWR International**	Phenol: Chloroform premixed with Isoamyl Alcohol 25:24:1	100513-510	100
VWR International**	Phenol: Chloroform Buffered Solution 25:24:1	IB05174	400

*www.amresco-inc.com or (US) 800.829.2802
** www.vwr.com or (US) 800.932.5000
* **International customers should contact their local distributor

Experienced User Protocol

Wear RNase-Free gloves (1555) at all times and remove RNase from the work area using Lab Cleaner (12095) for RNase Removal. Both of these products are available from MO BIO. Please see "Other Quality Products Available" section at the end of this manual.

1. Add up to 2 g of soil to the 15 ml **Bead Tube** (provided). **Note:** Please refer to Hints and Troubleshooting Guide for information regarding the amount of soil to process.
2. Add 2.5 ml of **Bead Solution** to the **Bead Tube** and vortex to mix.
3. Add 0.25 ml of **Solution SR1** to the **Bead Tube** and vortex to mix.
4. Add 0.8 ml of **Solution SR2** and place the Bead Tube on the Vortex Adapter (MO BIO Catalog # 13000-V1-15 for Vortex Genie 2 or 13000-LV2-15 for Labnet Vortex) and vortex at maximum speed for 5 minutes.
5. Remove the **Bead Tube** from the Vortex Adapter and add 3.5 ml of phenol: chloroform:isoamyl alcohol (pH 6.5 – 8.0, [User supplied]) and vortex to mix until the biphasic layer disappears.
6. Place the **Bead Tube** on the Vortex Adapter and vortex at maximum speed for 10 minutes.
7. Remove the **Bead Tube** from the Vortex Adapter and centrifuge at 2500 x *g* for 10 minutes at room temperature.
8. Remove the **Bead Tube** from the centrifuge and carefully transfer the upper aqueous phase (avoiding the interphase and lower phenol layer) to a clean **15 ml Collection Tube** (provided). The thickness of the interphase will vary depending on the type of soil used. Discard the phenol:chloroform:isoamyl alcohol in an approved waste receptacle. **Note**: The biphasic layer will be thick and firm in soils high in organic matter and may need to be pierced to remove the bottom phenol layer.
9. Add 1.5 ml of **Solution SR3** to the aqueous phase and vortex to mix. Incubate at 4°C for 10 minutes.
10. Centrifuge at 2500 x g for 10 minutes at room temperature.
11. Transfer the supernatant, without disturbing the pellet, to a new **15 ml Collection Tube** (provided).
12. Add 5 ml of **Solution SR4** to the Collection Tube containing the supernatant, invert or vortex to mix, and incubate at -20°C for 30 minutes.
13. Centrifuge at 2500 x *g* for 30 minutes at room temperature.
14. Decant the supernatant and invert the **15 ml Collection Tube** on a paper towel for 5 minutes. **Note**: Depending on soil type, the pellet may be large and/or dark in color.
15. Add 1 ml of **Solution SR5** to the **15 ml Collection Tube** and resuspend the pellet completely. (**Note**: Depending on the soil type, the pellet may be difficult to resuspend. Resuspension may be aided by placing the tubes in a heat block or water bath at 45°C for 10 minutes, followed by vortexing. Repeat until the pellet is resuspended.)
16. Prepare one **RNA Capture Column** (provided) for each RNA Isolation Sample:
    a. Remove the cap of a **15 ml Collection Tube** (provided) and place the **RNA Capture Column** inside the **15 ml Collection Tube**. The column will hang in the **15 ml Collection Tube**.
    b. Add 2 ml of **Solution SR5** to the **RNA Capture Column** and allow it to gravity flow through the column and collect in the **15 ml Collection Tube**. Allow **Solution SR5** to completely flow through the column (**Optional**: The Collection Tube may be emptied after Solution SR5 has completely flowed through the column. **Note**: DO NOT ALLOW THE COLUMN TO DRY OUT PRIOR TO LOADING THE RNA ISOLATION SAMPLE.)
17. Add the RNA Isolation Sample from Step 15 onto the **RNA Capture Column** and allow it to gravity flow through the column. Collect the flow through in the **15 ml Collection Tube**.
18. Wash the column with 1 ml of **Solution SR5**. Allow it to gravity flow and collect the flow through in the **15 ml Collection Tube**.

19. Transfer the **RNA Capture Column** to a new **15 ml Collection Tube** (provided) and add 1 ml of **Solution SR6** to the **RNA Capture Column** to elute the bound RNA into the **15 ml Collection Tube**. Allow **Solution SR6** to gravity flow into the **15 ml Collection Tube**. Note: A new kit is available for DNA elution. See ***DNA Elution Procedure in the Hints and Troubleshooting Guide*** *or* contact MO BIO for details at technical@mobio.com
20. Transfer the eluted RNA to a **2.2 ml Collection Tube** (provided) and add 1 ml of **Solution SR4**. Invert at least once to mix and incubate at -20°C for 10 minutes.
21. Centrifuge the **2.2 ml Collection Tube** at 13,000 x *g* for 15 minutes at room temperature to pellet the RNA.
22. Decant the supernatant and invert the **2.2 ml Collection Tube** onto a paper towel for 10 minutes to air dry the pellet.
23. Resuspend the RNA pellet in 100 µl of **Solution SR7**. (**Note**: Although DNA carryover does not occur with the majority of soil types, certain soils high in organic matter may present unique carryover situations. In situations where the absence of DNA contamination is critical, the purified RNA should be tested for potential DNA carryover by performing PCR with qualified primers on the isolated RNA without performing prior reverse transcription amplification. The absence of a detectable amplification fragment by agarose electrophoresis indicates the absence of detectable carryover DNA. In the event DNA is detected, DNase treatment of the isolated RNA is recommended; see Additional Information Section for instruction).

Thank you for choosing the RNA PowerSoil™ Total RNA Isolation Kit.

Detailed Protocol (Describes what is happening at each step)

Wear RNase-Free gloves (1555) at all times and remove RNase from the work area using Lab Cleaner (12095) for RNase Removal. Both of these products are available from MO BIO. Please see "Other Quality Products Available" section at the end of this manual.

1. Add up to 2 g of soil to the 15 ml **Bead Tube** (provided). **Note:** Please refer to Hints and Troubleshooting Guide for information regarding the amount of soil to process.

2. Add 2.5 ml of **Bead Solution** to the **Bead Tube** and vortex to mix.

3. Add 0.25 ml of **Solution SR1** to the **Bead Tube** and vortex to mix.

   What's happening: The Bead Solution is a buffer used to disperse cells and soil particles. Solution SR1 contains SDS and other disruption agents which aid in complete cell lysis. In addition to aiding in cell lysis, SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of several organisms. ***Note:*** *If it gets cold, it will form a white precipitate. Heating to 60ºC will dissolve the SDS and will not harm the other disruption agents.*

4. Add 0.8 ml of **Solution SR2** and place the Bead Tube on the Vortex Adapter (MO BIO Catalog # 13000-V1-15 for Vortex Genie 2 or 13000-LV2-15 for Labnet Vortex) and vortex at maximum speed for 5 minutes.

   What's happening: Solution SR2 is a precipitation reagent used to remove non-DNA organic and inorganic material including humic substances, cell debris, and proteins. Contaminating organic and inorganic matter may reduce DNA purity and inhibit downstream DNA applications. Vortexing is critical for homogenization and cell lysis.

5. Remove the **Bead Tube** from the Vortex Adapter and add 3.5 ml of phenol: chloroform:isoamyl alcohol (pH 6.5 – 8.0, [User supplied]) and vortex to mix until the biphasic layer disappears.

6. Place the **Bead Tube** on the Vortex Adapter and vortex at maximum speed for 10 minutes.

   What's happening: Cells are lysed by combination of chemical agents from steps 1-5 and the mechanical shaking introduced by vortexing. Phenol:chloroform:Isoamyl alcohol is added to maximize lysing efficiency and yield. Lysed cell components are trapped in the solvent and proteins are denatured leaving the nucleic acid in solution.

   The MO BIO Vortex Adapter is designed to be a simple cost effective platform to facilitate the homogenization and cell lysis of samples. An alternative to the MO BIO Vortex Adapter would be to attach your tubes to your platform with tape. Note that tape can become loose and may result in uneven shaking and lysis efficiency resulting in inconsistent results or lower yields.

7. Remove the **Bead Tube** from the Vortex Adapter and centrifuge at 2500 x *g* for 10 minutes at room temperature.

   What's happening: Centrifugation results in phase separation of the sample mixture. Three phases will be visible after centrifugation. The lower organic phase containing proteins and cellular debris, the interphase containing humics and other organic and non-organic material, and the upper aqueous phase containing total nucleic acid. ***Note:*** *The thickness of the interphase will depend on the sample type. Samples high in organic content will have a thicker interphase.*

8. Remove the **Bead Tube** from the centrifuge and carefully transfer the upper aqueous phase (avoiding the interphase and lower phenol layer) to a clean **15 ml Collection Tube** (provided). The thickness of the interphase will vary depending on the type of soil used. Discard the phenol:chloroform:isoamyl alcohol in an approved waste receptacle. **Note**: The biphasic layer will be thick and firm in soils high in organic matter and may need to be pierced to remove the bottom phenol layer for disposal.

   What's happening: The upper aqueous phase containing the total nucleic acids from the sample is transferred to a new tube. Cellular debris, proteins, and organic material are left behind. ***Note:*** *Take care not to transfer material from the lower phase or the interphase.*

9. Add 1.5 ml of **Solution SR3** to the aqueous phase and vortex to mix. Incubate at 4°C for 10 minutes.

   What's happening: Solution SR3 is a secondary precipitation step to further remove proteins and cellular debris.

10. Centrifuge at 2500 x g for 10 minutes at room temperature.

11. Transfer the supernatant, without disturbing the pellet, to a new **15 ml Collection Tube** (provided).

    What's happening: The supernatant containing nucleic acids are transferred to a new 15 ml tube. Non-nucleic acid material is left behind.

12. Add 5 ml of **Solution SR4** to the Collection Tube containing the supernatant, invert or vortex to mix, and incubate at -20°C for 30 minutes.

13. Centrifuge at 2500 x *g* for 30 minutes at room temperature.

14. Decant the supernatant and invert the **15 ml Collection Tube** on a paper towel for 5 minutes. **Note**: Depending on soil type, the pellet may be large and/or dark in color.

    What's happening: Solution SR4 is 100% Isopropanol. Nucleic acid is precipitated and the Isopropanol is discarded.

15. Add 1 ml of **Solution SR5** to the **15 ml Collection Tube** and resuspend the pellet completely. (**Note**: Depending on the soil type, the pellet may be difficult to resuspend. Resuspension may be aided by placing the tubes in a heat block or water bath at 45°C for 10 minutes, followed by vortexing. Repeat until the pellet is resuspended.)

    What's happening: Solution SR5 is a proprietary salt solution used to resuspend the precipitated nucleic acids from step 14. It is also used to equilibrate the RNA capture column in step 16 and to wash and prep the column for the elution of RNA in step 20 below.

16. Prepare one **RNA Capture Column** (provided) for each RNA Isolation Sample:
    a. Remove the cap of a **15 ml Collection Tube** (provided) and place the **RNA Capture Column** inside the **15 ml Collection Tube**. The column will hang in the **15 ml Collection Tube**.
    b. Add 2 ml of **Solution SR5** to the **RNA Capture Column** and allow it to gravity flow through the column and collect in the **15 ml Collection Tube**. Allow **Solution SR5** to completely flow through the column (**Optional**: The Collection Tube may be emptied after

Solution SR5 has completely flowed through the column. **Note**: DO NOT ALLOW THE COLUMN TO DRY OUT PRIOR TO LOADING THE RNA ISOLATION SAMPLE.)

17. Add the RNA Isolation Sample from Step 15 onto the **RNA Capture Column** and allow it to gravity flow through the column. Collect the flow through in the **15 ml Collection Tube**.

18. Wash the column with 1 ml of **Solution SR5**. Allow it to gravity flow and collect the flow through in the **15 ml Collection Tube**.

    What's happening: The sample is added to the RNA Capture Column and the nucleic acids are bound to the column matrix. The Capture Column is then washed with a second volume of Solution SR5 to ensure unbound contaminants are removed from the sample and column prior to the elution of RNA.

19. Transfer the **RNA Capture Column** to a new **15 ml Collection Tube** (provided) and add 1 ml of **Solution SR6** to the **RNA Capture Column** to elute the bound RNA into the **15 ml Collection Tube**. Allow **Solution SR6** to gravity flow into the **15 ml Collection Tube**.

    What's happening: The Solution SR6 RNA elution buffer is a proprietary salt solution that allows for the preferential release of RNA from the RNA Capture Column leaving DNA, residual debris, and inhibiting substances in the column. Note: a new kit is available for DNA elution. See ***DNA Elution Procedure in the Hints and Troubleshooting Guide*** *or contact MO BIO for details at technical@mobio.com*

20. Transfer the eluted RNA to a **2.2 ml Collection Tube** (provided) and add 1 ml of **Solution SR4**. Invert at least once to mix and incubate at -20°C for 10 minutes.

21. Centrifuge the **2.2 ml Collection Tube** at 13,000 x *g* for 15 minutes at room temperature to pellet the RNA.

22. Decant the supernatant and invert the **2.2 ml Collection Tube** onto a paper towel for 10 minutes to air dry the pellet.

    What's happening: Solution SR4 is 100% Isopropanol. Eluted RNA from the Capture Column is precipitated, centrifuged, and allowed to air dry prior to resuspending and concentrating.

23. Resuspend the RNA pellet in 100 µl of **Solution SR7**.

    What's happening: Solution SR7 is RNase/DNase-Free water used to resuspend the pelleted DNA. Solution SR7 contains no EDTA. DNA is now ready for any downstream application. For long term storage of samples 10 mM Tris pH 8.0 or TE buffer may be used to reuspend the pelleted RNA.

(**Note**: Although DNA carryover does not occur with the majority of soil types, certain soils high in organic matter may present unique carryover situations. In situations where the absence of DNA contamination is critical, the purified RNA should be tested for potential DNA carryover by performing PCR with qualified primers on the isolated RNA without performing prior reverse transcription amplification. The absence of a detectable amplification fragment by agarose electrophoresis indicates the absence of detectable carryover DNA. In the event DNA is detected, DNase treatment of the isolated RNA is recommended; see Additional Information Section for instruction).

Thank you for choosing the RNA PowerSoil™ Total RNA Isolation Kit.

Hints and Troubleshooting Guide

Soil Types and Soil Amount to Process

The yield and purity of RNA will depend on the soil type processed. The RNA PowerSoil™ Total RNA Isolation Kit has been validated with diverse soil types that represent a wide range of physical, chemical and biological characteristics. In our experience, it is possible to use up to a maximum of 2 g for most soil types. For soils with high organic content, 1 g of soil typically gives an adequate amount of RNA while reducing the potential for DNA carryover during purification.

Phase Separation of Phenol:Chloroform:Isoamyl Alcohol

To ensure effective phenol:chloroform:isoamyl alcohol and aqueous phase separation, centrifuge 15 ml tubes at 2,500 x *g* at room temperature for at least 10 minutes (Step 7). Following centrifugation, the interphase thickness between the phenol:chloroform:isoamyl alcohol and aqueous layers will vary depending on the organic content of the soil. Care should be taken to avoid the lower phenol phase and the interphase containing protein and lipid when removing the aqueous upper phase. If a portion of the phenol layer or the interphase is removed, recentrifuge the transferred aqueous phase containing tube to obtain a phase separation that will permit removing the aqueous phase. Alternatively, an equal volume (2 ml) of chloroform may be added to the phenol or interphase contaminated aqueous phase, inverted or vortexed to mix, and the tubes centrifuged at 2,500 *x g* for 10 minutes at room temperature. Remove the upper aqueous phase and discard the lower chloroform:phenol interphase. Then combine with the rest of the aqueous phase and continue with protocol.

Pellet Resuspension in Solution SR5

Soil types with a high organic content may yield RNA pellets that are difficult to resuspend. Heating the RNA pellet in Solution SR5 at 45°C will aid in the resuspension process. Disrupting the RNA pellet with a pipette tip and vortexing vigorously will also aid in RNA resuspension. It is important to resuspend the pellet completely before applying it to the column in Step 17. Failure to completely resupend the RNA pellet will result in RNA loss through reduced column binding and will result in reduced column flow rate.

Column Flow

The RNA Capture Columns are rated for gravity flow and should not be used with centrifugal or vacuum force.

Suggested Protocol for Formaldehyde Agarose Gel Electrophoresis

Solutions Needed:

10x Formaldehyde Agarose Gel Buffer

200mM 3-[N-morpholino] propanesulfonic acid (MOPS, free acid)
50 mM Sodium Acetate
10 mM EDTA
pH to 7.0 with Sodium Hydroxide
Prevent exposure to light (store in a dark bottle).

1x Formaldehyde Agarose Gel Buffer (1L)

100 ml 10x Formaldehyde Agarose Gel Buffer
20 ml 37% (12.3 M) Formaldehyde
880 ml DEPC treated water

5x RNA Loading Dye

16 µl of Saturated Aqueous Bromophenol Blue Solution
80 µl of 5M EDTA, pH 8.0
720 µl of 37% (12.3M) Formaldehyde
2 ml of 100% Glycerol
3084 µl of Formamide
4 ml of 10x Formaldehyde Agarose Gel Buffer

Formaldehyde Agarose Gel Preparation

To make a 1.2% Formaldehyde Agarose Gel with 100 ml volume, mix the following:

1.2 g of Agarose
10 ml of 10x Formaldehyde Agarose Gel buffer
90 ml of DEPC treated water

Heat the mixture in a microwave oven to melt the agarose. Cool to 65°C in a water bath. Add 1.8 ml of 37% (12.3 M) formaldehyde and 2 µl of 5 mg/ml ethidium bromide solution. Swirl to mix and pour into a gel box (NOTE: Do not breath formaldehyde fumes. Pour the melted agarose in a fume hood or cover the gel tray with plastic wrap immediately after pouring.). Pre-run the gel for 30 minutes at 5 – 7 V/cm in 1x Formaldehyde Agarose Gel Buffer before loading the samples. After loading, run the gel at 5 – 7 V/cm until the bromophenol blue is approximately two-thirds of the distance to the gel edge (See RNA Sample Preparation).

RNA Sample Preparation

RNA analyzed by TAE or TBE native gel electrophoresis may be loaded and analyzed using native gel electrophoresis loading and gel running buffers.

RNA analyzed by formaldehyde agarose gel electrophoresis must be denatured before application to the gel. Add 1 volume of 5x RNA loading dye for each 4 volumes of RNA sample (i.e. 2 µl of 5x RNA loading dye for each 8 µl of RNA sample). Mix the sample and loading dye, and centrifuge to collect the sample at the bottom of the tube. Incubate the sample at 65°C for 3 – 5 minutes, chill on ice and centrifuge to collect the sample at the tube bottom. Load the sample into the Formaldehyde Agarose Gel and run the gel at 5-7 V/cm in 1x Formaldehyde Agarose Gel Buffer for approximately 90 minutes.

References

1. Beintema, J.J., Campagne, R.N., and Gruber, M. 1973. Biochim. Biophys. Acta 310: 148-160
Kaplan, B.B., Bernstein, S.L., and Gioio, A.E. 1979. Biochem. J. 183-181-184.

Digesting RNA with RNase-Free DNase

Note: ONLY RNase-Free DNase may be used with this protocol. The presence of RNases will result in digested RNA.

The presence of carryover DNA with RNA isolated using the RNA PowerSoil™ Total RNA Isolation Kit does not occur with the majority of soil types. It has been noted, however, that soils with high organic matter content may contain carryover DNA co-extracted with the isolated RNA. The following protocol, used in conjunction with the manufacturers' instructions, should serve as a guide to DNase digesting RNA.

a. If using the entire RNA sample, add the appropriate amount of DNase buffer, water and up to 4 Units of DNase to the RNA sample to obtain a total volume of 200 µl. A typical 10X DNase digestion buffer is 10 mM $CaCl_2$ and 10 mM $MgCl_2$ in Tris-HCl buffer, pH 7.5.
b. Incubate at 37°C for 30 to 45 minutes.
c. Add 200 µl of phenol:chloroform:isoamyl alcohol (pH 6.5 – 8.0) and vortex to mix. Incubate at room temperature for 5 minutes.

d. Centrifuge the sample at 10,000 x g for 5 minutes.
e. Carefully remove the upper aqueous phase and transfer it to another tube.
f. Add 1/10th volume of 5M NaCl, two volumes of 100% ethanol and invert to mix.
g. Incubate at -20°C for 30 minutes and centrifuge at 10,000 x g for 10 minutes.
h. Decant the supernatant and air dry the pellet.
i. Resuspend the pellet in an appropriate volume of Solution SR7. The RNA can be used directly in an RT-PCR reaction without dilution.

Multiple Elutions from the Same Column

Multiple elutions beyond those called for in the protocol are not recommended when using the Capture Columns. Although a small amount of additional RNA or DNA (if using the RNA PowerSoil™DNA Elution Accessory Kit, catalog #12867-25) may come off the column with multiple elutions, the inhibitors associated with the starting material will also begin to wash off the column and could cause inhibition in downstream applications.

Co-Isolation of DNA from the RNA Capture Column

After the RNA is eluted from the RNA capture column the DNA may also be eluted from the same column using the RNA PowerSoil™ DNA Elution Accessory Kit (MO BIO Catalog # 12867-25). The capture column is placed in a new 15 ml tube and the DNA elution buffer is added to the column and the DNA is eluted in a separate tube.

DNA Elution Procedure

(RNA PowerSoil™ DNA Elution Accessory Kit Catalog #12867-25 Required)

1. Transfer the RNA Capture Column from step 19 of the RNA PowerSoil™ Total RNA Isolation Kit (catalog #12866-25) to a 15 ml Collection Tube (provided) and add 1 ml of Solution SR8 to the Capture Column to elute the bound DNA into the 15 ml Collection Tube. Allow Solution SR8 to gravity flow into the 15 ml Collection Tube.
2. Transfer the eluted DNA to a 2.2 ml Collection Tube (provided) and add 1 ml of Solution SR4. Invert at least once to mix and incubate at -20°C for 10 minutes.
3. Centrifuge the 2.2 ml Collection Tube at 13,000 x g for 15 minutes at room temperature to pellet the DNA.
4. Decant the supernatant and invert the 2.2 ml Collection Tube onto a paper towel for 10 minutes to air dry the pellet.
5. Resuspend the DNA pellet in 100 µl of Solution SR7.

Contact Information

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For the distributor nearest you, visit our web site at www.mobio.com/distributors

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DNA Purification and Gel Extraction	Catalog No.	Quantity
PowerClean™ DNA Clean-Up Kit	12877-50	50 preps
UltraClean™ 15 DNA Purification Kit	12100-300	300 preps
UltraClean™ PCR Clean-Up Kit	12500-50 12500-100 12500-250	50 preps 100 preps 250 preps
UltraClean-htp™ 96 Well PCR Clean-Up Kit	12596-4 12596-12	4 x 96 preps 12 x 96 preps
UltraClean™ GelSpin™ DNA Extraction Kit	12400-50 12400-100 12400-250	50 preps 100 preps 250 preps
Plasmid DNA Isolation	**Catalog No.**	**Quantity**
UltraClean™ 6 Minute Mini Plasmid Prep Kit	12300-50 12300-100 12300-250	50 preps 100 preps 250 preps
UltraClean™ Standard Mini Plasmid Prep Kit	12301-50 12301-100 12301-250	50 preps 100 preps 250 preps
UltraClean-htp™ 96 Well Plasmid Prep Kit	12396-4 12396-12	4 x 96 preps 12 x 96 preps
UltraClean™ Midi Plasmid Prep Kit	12700-20 12700-50	20 preps 50 preps
UltraClean™ Maxi Plasmid Prep Kit	12600-10 12600-20	10 preps 20 preps
UltraClean™ Endotoxin-Free Mini Plasmid Prep Kit	12311-100 12311-250	100 preps 250 preps
UltraClean™ Endotoxin-Free Midi Plasmid Prep Kit	12711-10	10 preps
UltraClean™ Endotoxin-Free Maxi Plasmid Prep Kit	12611-10	10 preps
UltraClean™ Endotoxin Removal Kit	12615	1 kit
UltraClean™ Endotoxin-Free Ethanol Precipitation Kit	12616	1 kit
UltraClean™ Endotoxin Removal Reagent	12625-25	25 ml
Endotoxin-Free Sodium Chloride	12626-15	15 ml
Endotoxin-Free Centrifuge Tubes	12617-100 12618-50 12619-25	100 each/2 ml tubes 50 each/15 ml tubes 25 each/50 ml tubes
RNA Isolation	**Catalog No.**	**Quantity**
RNA PowerSoil™ Total RNA Isolation Kit	12866-25	25 preps
UltraClean™ Microbial RNA Isolation Kit	15800-50 15800-250	50 preps 250 preps
UltraClean™ Tissue RNA Isolation Kit	15000-50 15000-250	50 preps 250 preps
UltraClean™ Plant RNA Isolation Kit	13300-20 13300-50	20 preps 50 preps
Genomic DNA Isolation	**Catalog No.**	**Quantity**
PowerMax™ Soil DNA Isolation Kit	12988-10	10 preps
PowerSoil™ DNA Isolation Kit	12888-50 12888-100	50 preps 100 preps

Genomic DNA Isolation…*Continued*	Catalog No.	Quantity
PowerSoil-htp™ 96 Well Soil DNA Isolation Kit	12955-4 12955-12	4 x 96 preps 12 x 96 preps
UltraClean™ Soil DNA Isolation Kit	12800-50 12800-100	50 preps 100 preps
UltraClean-htp™ 96 Well Soil DNA Isolation Kit	12896-4 12896-12	4 x 96 preps 12 x 96 preps
UltraClean™ Mega Soil DNA Isolation Kit	12900-10	10 preps
PowerClean™ DNA Clean-Up Kit	12877-50	50 preps
UltraClean™ Fecal DNA Isolation Kit	12811-50 12811-100	50 preps 100 preps
PowerMicrobial™ Midi DNA Isolation Kit	12225-25	25 preps
PowerMicrobial™ Maxi DNA Isolation Kit	12226-25	25 preps
UltraClean™ Microbial DNA Isolation Kit	12224-50 12224-250	50 preps 250 preps
UltraClean-htp™ 96 Well Microbial DNA Isolation Kit	10196-4 10196-12	4 x 96 preps 12 x 96 preps
PowerPlant™ DNA Isolation Kit	13200-50 13200-100	50 preps 100 preps
UltraClean™ Plant DNA Isolation Kit	13000-50 13000-250	50 preps 250 preps
UltraClean-htp™ 96 Well Plant DNA Isolation Kit	13096-4 13096-12	4 x 96 preps 12 x 96 preps
UltraClean™ Tissue DNA Isolation Kit	12334-50 12334-250	50 preps 250 preps
UltraClean-htp™ 96 Well Tissue DNA Isolation Kit	12996-4 12996-12	4 x 96 preps 12 x 96 preps
UltraClean™ Blood DNA Isolation Kit (Non-Spin)	12000-100	100 preps
UltraClean™ Blood DNA Isolation Kit (Processes 1,000 ml of Blood)	12000-1000	1 kit
UltraClean™ Blood DNA Isolation Kit Plus RNase (Processes 1,000 ml of Blood)	12002-1000	1 kit
UltraClean™ BloodSpin™ DNA Isolation Kit	12200-50 12200-250	50 preps 250 preps
UltraClean-htp™ 96 Well BloodSpin™ DNA Isolation Kit	12296-4 12296-12	4 x 96 preps 12 x 96 preps
UltraClean™ Mega BloodSpin™ DNA Isolation Kit	12210-10	10 preps
UltraClean™ Forensic DNA Isolation Kit	14000-10 14000-20	10 isolations 20 isolations
UltraClean™ Water DNA Isolation Kit (0.45µm filters)	14800-10 14800-25	10 preps 25 preps
UltraClean™ Water DNA Isolation Kit (0.22 µm filters)	14880-10 14880-25	10 preps 25 preps
UltraClean™ Water DNA Isolation Kit (no filters)	14800-10-NF 14800-25-NF	10 preps 25 preps

Other Quality Products Available from MO BIO Laboratories, Inc.

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Microbiological Culture Media	Catalog No.	Quantity
TB DRY™ Powder Growth Media	12105-05 12105-1 12105-5	500 g 1 kg 5 kg
LB Broth Powder Growth Media, pH 7	12106-05 12106-1 12106-5	500 g 1 kg 5 kg
LB Agar Powder Growth Media, pH 7	12107-05 12107-1 12107-5	500 g 1 kg 5 kg
LB Broth (Lennox) Powder Growth Media, pH 7	12108-05 12108-1 12108-5	500 g 1 kg 5 kg
LB Agar (Lennox) Powder Growth Media, pH 7	12109-05 12109-1 12109-5	500 g 1 kg 5 kg
Soybean-Casein Digest Medium (TSB), USP	12114-05 12114-1 12114-5	500 g 1 kg 5 kg
Soybean-Casein Digest Agar Medium (TSA), USP	12115-05 12115-1 12115-5	500 g 1 kg 5 kg
Yeast Extract	12110-05 12110-1 12110-5	500 g 1 kg 5 kg
Tryptone	12111-05 12111-1 12111-5	500 g 1 kg 5 kg
Agar, Bacteriological Grade	12112-05 12112-1 12112-5	500 g 1 kg 5 kg
Other Reagents and Lab Accessories	**Catalog No.**	**Quantity**
20 bp DNA Ladder	17020-40	40 µg
100 bp DNA Ladder	17100-40	40 µg
1 kb DNA Ladder	17200-100	100 µg
UltraClean™ Agarose, Molecular Biology Grade	15003-50 15003-100 15003-500 15003-1000	50 g 100 g 500 g 1 kg
UltraClean™ MS-8 Agarose	15515-50 15515-100 15515-500	50 g 100 g 500 g
UltraClean™ Forensic Agarose	15505-50 15505-100 15505-500	50 g 100 g 500 g
UltraClean™ Low Melt Agarose	15005-50 15005-100 15005-500	50 g 100 g 500 g
UltraClean™ Low Melt Sieve Agarose	15004-50 15004-100 15004-500	50 g 100 g 500 g
Ethidium Bromide Solution	15006-1 15006-10	1 ml 10 ml

Other Reagents and Lab Accessories…*Continued*	Catalog No.	Quantity
Ethidium Bromide Destaining Tea Bags	15007-25	25 bags
Bromophenol Blue Gel Loading Buffer	15008-1 15008-5	1 ml 5 x 1 ml
Bromophenol Blue/Xylene Cyanol Gel Loading Buffer	15009-1 15009-5	1 ml 5 x 1 ml
TAE Buffer, 50X (Tris-acetate-EDTA)	15001-100 15001-500 15001-1000	100 ml 500 ml 1 liter
TBE Buffer, 10X (Tris-borate-EDTA)	15002-100 15002-500 15002-1000	100 ml 500 ml 1 liter
RNase-Free Gloves	1555-XS 1555-S 1555-M 1555-L	bag of 100 bag of 100 bag of 100 bag of 100
UltraClean™ Lab Cleaner	12095-250 12095-500 12095-1000	250 ml squeeze bottle 500 ml spray bottle 1 liter bottle
OmniTaq™ DNA Polymerase Enzyme	1224-250	250 reactions (10 U/µl)
OmniTaq™ DNA Polymerase 2x Master Mix	1226-250	250 reactions (5 x 1.25 ml/tube)
Omni KlenTaq™ DNA Polymerase Enzyme	1225-250	250 reactions (25 U/µl)
Omni KlenTaq™ DNA Polymerase 2x Master Mix	1227-250	250 reactions (5 x 1.25 ml/tube)
DNase (RNase-Free)	15600-5	5 mg
Proteinase K	1223-100 1222-2	100 mg 2 ml (20 mg/ml)
Ribonuclease A	1202-1 1202-5	1 ml (25 mg/ml) 5 ml (25 mg/ml)
PCR Water	17000-1 17000-5 17000-10 17000-11	1 ml 5 x 1 ml 10 x 1 ml 10 ml bottle
Molecular Biology Grade Water	17012-200 17012-5200	200 ml 5 x 200 ml
DEPC Treated Water	17011-200 17011-5200	200 ml 5 x 200 ml
Endotoxin-Free Water	17013-10 17013-50 17013-100 17013-500	10 ml 50 ml 100 ml 500 ml

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Instrumentation and Accessories	Catalog No.	Quantity
Precellys® 24 Homogenizer, 120V	13112	1 unit
Ceramic Bead Tubes, 1.4 mm	13113-50	50 bead tubes
Ceramic Bead Tubes, 2.8 mm	13114-50	50 bead tubes
Glass Bead Tubes, 0.5 mm	13116-50	50 bead tubes
Glass Bead Tubes, 0.1 mm	13118-50	50 bead tubes
Metal Bead Tubes, 2.38 mm	13117-50	50 bead tubes
2.0 ml Tough Tubes with Cap	13119-500 13119-1000	500 x 2.0 ml Tough Tubes with Cap 1000 x 2.0 ml Tough Tubes with Cap
Carbide Bead Tubes, 0.25 mm	13121-50	50 x 0.5 ml tubes
Garnet Bead Tubes, 0.15 mm	13122-50	50 x 0.5 ml tubes
Garnet Bead Tubes, 0.70 mm	13123-50	50 x 2 ml tubes
2 ml Collection Tubes	1200-100-T 1200-150-T 1200-250-T	100 tubes 150 tubes 250 tubes
2 ml Screw Cap Tubes	12800-200-E	200 tubes & caps
15 ml Collection Tubes	12700-T	25 tubes
50 ml Centrifuge Tubes	12600-T	25 tubes
Spin Filters	1200-50-SF 1200-100-SF 1200-250-SF	50 filters in tubes 100 filters in tubes 250 filters in tubes
Endotoxin-Free Centrifuge Tubes	12617-100 12618-50 12619-25	100 each/2 ml tubes 50 each/15 ml tubes 25 each/50 ml tubes
15 ml Midi Spin Filters	12700-SF	25 spin filters
Vortex-Genie® 2 Vortex (120V)	13111-V	1 unit
Vortex-Genie® 2 Vortex (220V)	13111-V-220	1 unit
Vortex Adapter, holds 12 (1.5-2.0 ml) tubes	13000-V1	1 unit
Vortex Adapter, holds 4 (15 ml) tubes	13000-V1-15	1 unit

Instrumentation and Accessories…*Continued*	Catalog No.	Quantity
Vortex Adapter, holds 2 (50 ml) tubes	13000-V1-50	1 unit
Vortex Adapter, holds 24 (1.5-2.0 ml) tubes	13000-V1-24	1 unit
Power Supply w/Timer, (120V)	16023	1 unit
Power Supply w/Timer, (220V)	16023-220	1 unit
Polycarbonate Single-sided Comb	16005 16006 16007 16008	1 mm x 3 well 1 mm x 8 well 1 mm x 10 well 1 mm x 12 well
Polycarbonate Dual-sided Comb	16013 16014 16015 16016	1 mm x 8 well/16 well 1 mm x 10 well/14 well 2 mm x 8 well/16 well 2 mm x 10 well/14 well
Teflon Single-sided Comb	16009 16010 16011 16012	1 mm x 3 well 1 mm x 8 well 1 mm x 10 well 1 mm x 12 well
Teflon Dual-sided Comb	16017 16018 16019 16020	1 mm x 8 well/16 well 1 mm x 10 well/14 well 2 mm x 8 well/16 well 2 mm x 10 well/14 well
Mini Horizontal Gel System	16001	1 each
Mini Horizontal Gel Caster, 1 place	16002	1 each
Mini Horizontal Gel Caster, 3 place	16003	1 each
Mini Horizontal Gel Tray	16004	1 each
96 Well Plate Shaker (120V)	11996	1 unit
96 Well Plate Shaker (220V)	11996-220	1 unit
Plate Adapter Set	11999	1 set
Tube Adapter Set	11995	1 set
Vacuum Pump (120V)	11998	1 unit
Vacuum Pump (220V)	11998-220	1 unit
UltraVac™ Manifold	11997	1 unit